J. Appl. Ichthyol. 11 (1995), 225–230
© 1995 Blackwell Wissenschafts-Verlag, Berlin
ISSN 0175–8659

Received: October 20, 1994
Accepted: June 27, 1994

Requirements for carotenoids in fish diets

By O. J. Torrissen and R. Christiansen

Institute of Marine Research, Matre Aquaculture Station, N-5198 Matredal, Norway

Summary

Carotenoids are among the most widespread and important pigment classes in living organisms, and in marine animals, astaxanthin is the most commonly occurring red carotenoid. Carotenoids are vitamin A precursors and are fundamental in photosynthesis and light protection in plants. Increasing attention has been drawn to a possible light protection, cancer prevention and immune enhancement by carotenoids in mammals. Reported functions in fish range from a general enhancement of performance to specific functions in reproduction and metabolism. In this paper, we show that astaxanthin is essential for growth and survival of fish and crawfish, and discuss this fact in relation to the use of purified and semipurified diets in nutritional studies. The similarity in action of astaxanthin and canthaxanthin compared to α-tocopherol (vitamin E) and retinol (vitamin A) suggests that these two carotenoids should be listed among the fat soluble vitamins.

Introduction

Carotenoids in modern aquaculture are mainly associated with astaxanthin or canthaxanthin pigmentation of the flesh of salmonids. The pigmentation of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) flesh is regarded as the most important quality criteria next to product freshness (Koteng 1992). It is therefore of vital interest for the salmon farmer to achieve a satisfactory pigmentation of the salmon flesh. The market demand for astaxanthin in Atlantic salmon flesh requires a concentration above 6–7 mg per kg. Factors influencing the absorption and deposition of carotenoids are reviewed by Torrissen et al. (1989) and Storebakken and No (1992).

It has been shown that carotenoids have at least four functions: (1) accessory pigment in photosynthesis, (2) protective pigment against photosensitization, (3) provitamin A source and (4) communication in aquatic animals. Responses to physiological or pharmacological administration of carotenoids are normally classified as actions. Potential mechanisms associated with carotenoid actions include antioxidant and singlet oxygen quenching, provitamin A activity, up-regulation of DNA expression, co-oxidation, and enhancement of immune functions associated with increased tumour immunity and modulation of macrophage and lymphocyte activation (Bendich 1993).

Salmonids absorb and deposit astaxanthin and canthaxanthin in the muscle during the grow out period. At the time of sexual maturation, they mobilize the carotenoid store and transport the accumulated astaxanthin or canthaxanthin by the Very High Density Lipoproteins (VHDL) (vitellogenin) or High Density Lipoproteins (HDL) to the ovaries and finally the progeny. This active transfer of carotenoids from the mother fish to the eggs has led to the hypothesis that carotenoids are vital for egg and larval development.

Absorption and storage

Some carotenoids are absorbed well and rapidly metabolized, some are absorbed and poorly metabolized and some seems not to be absorbed at all. Both the absorption and metabolism of carotenoids are highly dependent on its structure. The mechanism by which the carotenoids are taken up from the plasma by tissues and released back into plasma from specific

U. S. Copyright Clearance Center Code Statement: 0175–8659/95/1104–0225 $11.00/0

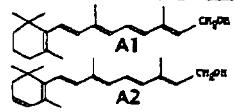
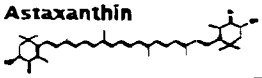
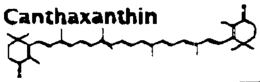
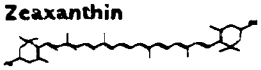
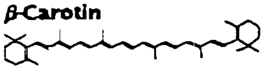
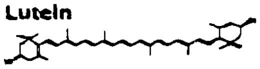
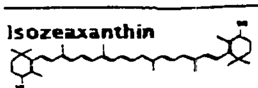
Carotenoid	Absorbed	Growth	
Astaxanthin 	*****	Yes	A1 & A2
Canthaxanthin 	***	Yes	A1 & A2
Zeaxanthin 		Yes	A1 & A2
β-Carotin 	*	Yes	A1
Lutein 		Yes	A2
Isozeaxanthin 		Yes	A2

Fig. 1. Main carotenoids used in fish nutrition and their structures; A1 = retinol; A2 = dehydroretinol; *, ***, ***** indicates magnitude of difference in absorbtion

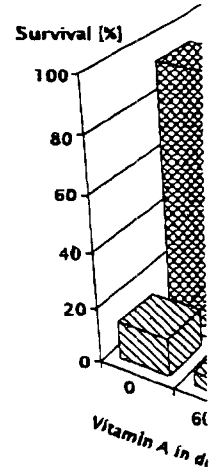
tissues, however, has not yet been clarified (OLSON 1993). A preferred absorption and deposition of hydroxy and keto carotenoids is seen in fish. Astaxanthin and canthaxanthin are absorbed well, while β-carotene in some fishes seems hardly to be absorbed at all.

Growth and survival

An improved growth of Atlantic salmon was found by supplementing commercial start feeding diets with astaxanthin or canthaxanthin (Fig. 1) (TORRISSEN 1984), and no significant differences were found between the astaxanthin and canthaxanthin supplemented diets.

Corresponding results are found for the red tilapia (*Oreochromis niloticus*) (BOONYARATPALIN and UNPRASERT 1989), and kuruma shrimp (*Penaeus japonicus*) (CHIEN and JENG 1992). Supplementation of β-carotene and canthaxanthin to the diets of major Indian carps resulted in a better survival and growth compared to conventional diets used without carotenoids (GOSWAMI 1993), and NÈGRE-SADARGUES et al. (1993) found a higher survival rate for *Penaeus japonicus* receiving astaxanthin-canthaxanthin supplementation (50/50), but no differences were observed in growth and moulting.

CHRISTIANSEN et al. (1993) investigated the interaction between astaxanthin and vitamin A supplementation on growth and survival in first feeding fry of Atlantic salmon. The experimental diets were based on a semipurified diet based on vitamin and carotenoid free casein and gelatine as protein sources developed by SHEARER et al. (1993). The results from this 135-day feeding study clearly showed a significantly improved growth and survival on supplementation of astaxanthin to the experimental diet (Figs 2 and 3), and vitamin A supplementation alone did not support growth and survival. The vitamin A source used was a mixture of retinol palmitate and retinol acetate. The bio-availability of the two forms



is not known and
also show a provi
explain the effect

GROSS and BUDO
addition to β-carot
platies (*Xiphophon*
both A₁ and A₂ in
highly dependent
(1985) reported th
A in the intestinal
batrachus, lutein (
(dehydroretinol) (
tilapia (*Tilapia nil*
converted into vita

Fishes change the
responses during
as compromises b
the 'need' to avoi
changes is comple
visual cues. It has
and xanthophores
(1952) suggested
pigments may hav

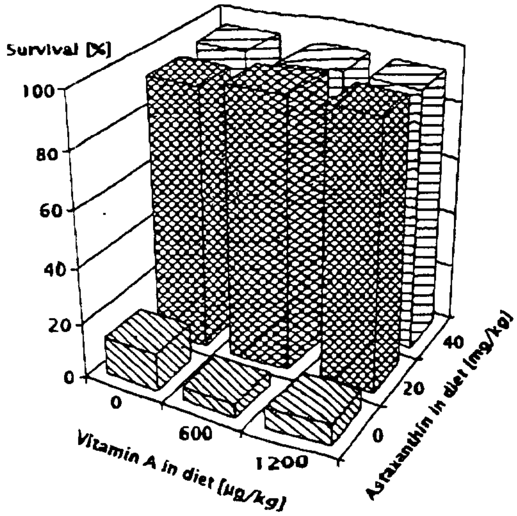


Fig. 2. Survival of Atlantic salmon fed diets supplemented with different levels of astaxanthin and vitamin A (CHRISTIANSEN et al. 1993)

is not known and one or both might be poorly utilized by start-feeding salmon. The results also show a provitamin A function of astaxanthin (Fig. 4), but this alone is not able to explain the effect of astaxanthin supplementation.

Functions of carotenoids

Provitamin A

GROSS and BUDOWSKI (1966) reported that astaxanthin, canthaxanthin and isozeaxanthin in addition to β-carotene were precursors for vitamin A in both guppies (*Lebistes reticulatus*) and platies (*Xiphophorus variatus*). Astaxanthin, canthaxanthin and zeaxanthin were precursors of both A_1 and A_2 in rainbow trout (*Oncorhynchus mykiss)*, but the rate of incorporation was highly dependent on fish size and age, and the vitamin A status of the fish. SCHIEDT et al. (1985) reported that astaxanthin, canthaxanthin and zeaxanthin were transformed into vitamin A in the intestinal wall and the liver. In the freshwater fishes, *Saccobranchus fossilis* and *Clarias batrachus*, lutein (3,3' Dihydroxy α-carotene) is reported to be the precursor of vitamin A_2 (dehydroretinol) (BARUA and GOSWAMI 1977; GOSWAMI and BHATTACHARJEE 1982), and in tilapia (*Tilapia nilotica*) astaxanthin, zeaxanthin, lutein and tunaxanthin were directly bio-converted into vitamin A(2) (KATSUYAMA and MATSUNO 1988).

Communication

Fishes change their hues in response to background colouration and also display colour responses during excitement and courtship (FUJII 1969). The colour pattern can be viewed as compromises between the 'need' to communicate with other members of the species and the 'need' to avoid being eaten (MOYLE and CECH 1982). The internal control of colour changes is complex and involves both hormones and nerves where the initiation comes from visual cues. It has been shown that carotenoids are integral constituents of chromatophores and xanthophores, and, as such, are functional in the photo-responses of fish. GOODWIN (1952) suggested this to be the major role of carotenoids in fish, and lack of sufficient pigments may have a negative effect on their general performance.

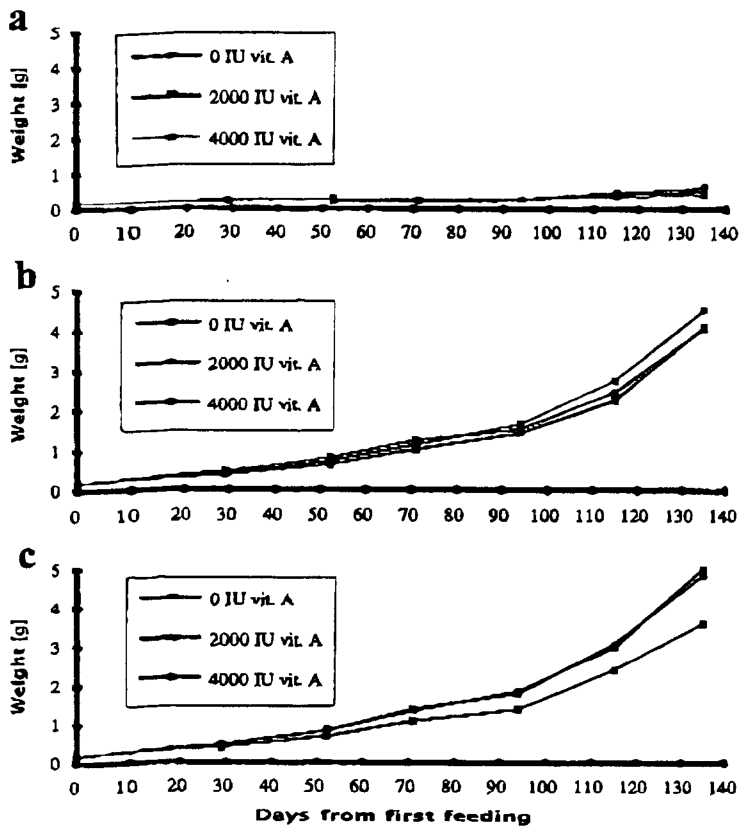


Fig. 3. Growth of Atlantic salmon fry fed diets supplemented with different concentrations of vitamin A and astaxanthin. IU = international units; (a) Fish fed semi-purified diets free of Astaxanthin; (b) Semi-purified diets supplemented with 20 ppm Astaxanthin; (c) Semi-purified diets supplemented with 40 ppm Astaxanthin (CHRISTIANSEN et al. 1992)

Actions of carotenoids

TVERANGER (1984) did not detect any effect of astaxanthin supplementation on fertility of rainbow trout, however, improved egg buoyancy was observed from red sea bream (*Chrysophrys major*) broodstock fed diets containing β-carotene, canthaxanthin and astaxanthin the night before spawning. Hatching was not affected, but the number of oil globules was reduced. CHRISTIANSEN and TORRISSEN (unpublished data) did not find any effect of astaxanthin content on the hatching success of Atlantic salmon eggs.

Heteropneustes fossilis showed atrophied gonads with damaged germinal epithelium when fed a carotenoid-free diet (GOSWAMI 1988). SENGER et al. (1989) reported an improved liver histology in *Oreochromis niloticus* and *Colisa labiosa* fed high astaxanthin levels (71–132 g/kg) and low level (32 mg/kg) in the diet. Particularly the parenchymal and intracellular organization was better developed. In tilapia the glycogen storage was enhanced and the cell volume slightly increased, although the biochemical mechanism is unknown.

Marine pelagic cold water fish spawn large numbers of small eggs without visible carotenoid depositions, while demersal fish and viviparous fish often have eggs containing high levels of carotenoids. Pelagic eggs have, in general, a short period of development from

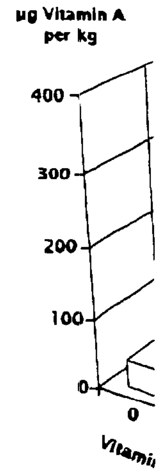


Table 1. Formu
and vitamin A

Ingredients
Casein
Fishoil (Sar
Gelatin
Dextrin
Carboxyme
α-cellulose

Amino acids
L-arg
L-his
L-lys
L-met
L-phe
L-thr

[1]The vitamin
vitamin D_3; 1(
riboflavin; 15
acid; 40 mg bi
ml/kg dry ing
$NaHSeO_3$; 4.0

spawning unt
satisfies their
may be an ad
halibut and tu
predominant l
supplies of vit
that vitamin A
punctatus). Th

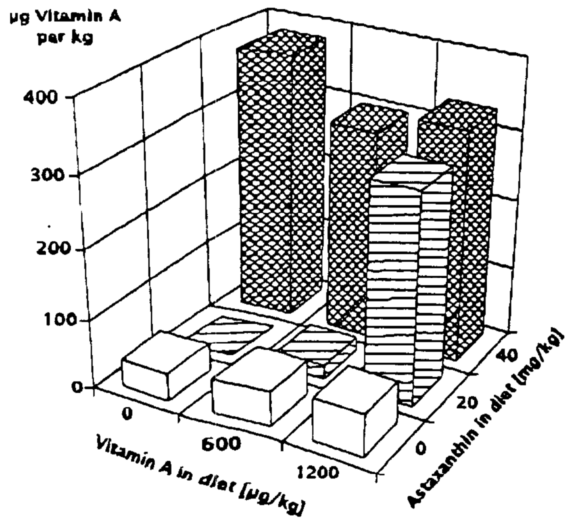


Fig. 4. Vitamin A content of Atlantic salmon fry fed diets supplemented with vitamin A and astaxanthin

Table 1. Formulation of a semipurified test diet for Atlantic salmon. Concentrations of astaxanthin and vitamin A mg/g dry diet. All other ingredients are given as percentage (SHEARER et al. 1993)

Ingredients			
Casein	44.8		
Fishoil (Sardine)	17.1	Vitamin mix[1]	2.0
Gelatin	10.0	Vitamin A [µg]	0, 600, 1200
Dextrin	12.0	Astaxanthin [µg/g]	0, 20, 40
Carboxymethylcellulose	1.0	Choline Chloride	1.0
α-cellulose	4.6		
Amino acids		KCl	1.5
L-arg	1.0	NaCl	0.3
L-his	0.2	$CaHPO_4 * H_2O$	1.2
L-lys	1.0	MgO	0.3
L-met	0.4	Trace min. sol.[2]	
L-phe	0.5		
L-thr	1.0		

[1] The vitamin mix contained the following vitamins per kg of diet: 1.188 g ascorbic acid; 4 mg vitamin D_3; 100 mg α-tocopherol acetate; 6 mg vitamin K_3; 15 mg thiamin hydrochloride; 30 mg riboflavin; 15 mg pyridoxine hydrochloride; 45.5 mg calcium D-phantothenat; 150 mg nicotinic acid; 40 mg biotin (2%); 4 mg folic acid; 3 mg vitamin B_{12} (1%); 300 mg inositol; [2] Supplied as 100 ml/kg dry ingredients contain: 1.9 mg KI; 32.5 mg $MnSO_4\ H_2O$; 88.0 mg $ZnSO_4\ 7H_2O$; 4.2 mg $NaHSeO_3$; 4.0 $CoCl_2\ 6H_2O$; 11.8 mg $CuSO_4\ 5H_2O$

spawning until the progeny start exogenous feeding (phyto- and zooplankton) which satisfies their carotenoid requirement. The lack of visible carotenoids in transparent eggs may be an adaptation to minimize predation pressure. Abnormal skin pigmentation of halibut and turbot is a large problem in first feeding of larvae on rotifers and artemia. The predominant hypothesis is that unpigmented skin is a deficiency syndrome for insufficient supplies of vitamin A and highly unsaturated fatty acids (HUFA). It has also been shown that vitamin A deficiency causes a depigmentation of the skin of channel catfish (*Ictalurus punctatus*). This deficiency syndrome seems to be reduced by enriching the live food with carotenoid containing algae. We have shown that vitamin A esters have a limited availability for Atlantic salmon fry (CHRISTIANSEN et al. 1993), and pilot studies indicate that marine fish larvae require carotenoids or preferably astaxanthin.

Diet supplementation

It is shown in a series of investigations on a wide variety of fishes and crayfish that supplementation of carotenoids to the diet improves growth, reduces the mortality rate and enhances the general performance of the animal. In addition a large amount of empirical data suggests that a sufficient carotenoid supply is essential for the well-being of the animal.

Astaxanthin or canthaxanthin should be regarded as a vitamin for fish and crawfish and added to all fish diets at a level above 10 mg kg^{-1} dry diet.

References

BARUA, A.B.; GOSWAMI, U.C., 1977: Formation of Vitamin A in a freshwater fish. Isolation of retinoic acid. Biochem. J. **164**: 133–136.

BENDICH, A., 1993: Recent advances in clinical research involving carotenoids. 10th Int. Symp. Carotenoids, Trondheim, 20–25 June, 1993, Book of Abstracts, SL-9.

BOONYARATPALIN, M.; UNPRASERT, N., 1989: Effect of pigments from different sources on colour changes and growth of red *Oreochromis niloticus*. Aquaculture **79**: 375–380.

CHIEN, Y.H.; JENG, S.C., 1992: Pigmentation of kuruma prawn, *Penaeus japonicus* Bate, by various pigment sources and levels and feeding regimes. Aquaculture **102**: 33–346.

CHRISTIANSEN, R.; LIE, Ø.; TORRISSEN, O.J., 1993: Effect of astaxanthin and vitamin A on growth and survival during first feeding of Atlantic salmon *Salmo salar* L. Aquaculture and Fisheries Management (in press).

FUJII, R., 1969: Chromatophores and pigments In: HOAR, W.S.; RANDALL, D.J. (eds), Fish Physiology, Vol. III, pp. 307–353.

GOODWIN, T.W., 1952: The Comparative Biochemistry of the Carotenoids. Chapman and Hall, London.

GOSWAMI, U. C., 1988: Carotenoid-free diet and its role in reproduction of fresh water fish. 8th Int. Symp. Carotenoids, Boston, 27–31 July, 1988, Abstracts of Presentations 32.

GOSWAMI, U.C., 1993: Metabolism of carotenoids in freshwater fish: (i) Biogenesis of 3-4 dehydroretinol (ii) supplementation of carotenoids with fish food for better survival and growth. 10th Int. Symp. Carotenoids, Trondheim, 20–25 June 1993, Book of Abstracts, CL 10–7.

GOSWAMI, U. C.; BHATTACHARJEE, S., 1982: Biosynthesis of 3-dehydroretinol: metabolism of lutein (β-carotene-3, 3′ Diol) in *Clarias batrachus* and *Ompok pabo*. Biochem. Int. **5**: 545–552.

GROSS, J.; P. BUDOWSKI, 1966: Conversion of carotenoids into Vitamin A_1 and A_2 in two species of freshwater fish. Biochem. J. **101**: 747–754.

KATSUYAMA, M.; T. MATSUNO, 1988: Carotenoid and vitamin A, and metabolism of carotenoids, beta-carotene, canthaxanthin, astaxanthin, zeaxanthin, lutein and tunaxanthin in tilapia *Tilapia nilotica*. Comp. Biochem. Physiol. **90B**: 131–139.

KOTENG, D.F., 1992: Markedsundersøkelse norsk laks. FNL, Bergen.

MOYLE, P.B.; CECH, J.J. JR., 1982: Fishes: an Introduction to Ichthyology, pp. 162–168. Prentice-Hall Inc., Englewood Cliffs, New Jersey.

NÈGRE-SADARGUES, G.; CASTILLO, R.; PETIT, H.; SANCE, S.; GOMEZ, R.; MILICUS, J.C.; CHOUBERT, G.; TRILLES, J.P., 1993: Utilisation of synthetic carotenoids by the prawn *Penaeus japonicus* under experimental rearing conditions. 10th Int. Symp. Carotenoids, Trondheim 20–25 June 1993, Book of Abstracts, CL 10–3.

OLSON, J. A., 1993: Absorption, transport, and metabolism of carotenoids in humans. 10th Int. Symp. Carotenoids, Trondheim, 20–25 June 1993, Book of Abstracts, SL 3.

SCHIEDT, K.; LEUENBERGER, F.J.; VECCHI, M.; GLINZ, E., 1985: Absorption retention and metabolic transformations of carotenoids in rainbow trout salmon and chicken. Pure and Applied Chemistry **57**: 685–692.

SEGNER, H.; AREND, P.; VON POEPPINGHAUSEN, K.; SCHMIDT, H., 1989: The effect of feeding astaxanthin to *Oreochromis niloticus* and *Colisa labiosa* on the histology of the liver. Aquaculture **79**: 381–390.

SHEARER, K.D.; CHRISTIANSEN, R.; DAAE, B.; TORRISSEN, O.J., 1993: Evaluation of four semipurified protein sources for use in juvenile Atlantic salmon (*Salmo salar*) test diets. Proc. IV Int. Symp. Nutrition and Feeding in Fish (in press).

STOREBAKKEN, T.; NO, H.K., 1992: Pigmentation of rainbow trout. Aquaculture **100**: 209–229.

TORRISSEN, O.J., 1984: Pigmentation of salmonids — effect of carotenoids in eggs and startfeeding diet on survival and growth rate. Aquaculture **43**: 185–193.

TORRISSEN, O.J.; HARDY, R.W.; SHEARER, K.D., 1989: Pigmentation of salmonids — carotenoid deposition and metabolism. CRC Crit. Rev. in Aquat. Sci. **1**: 209–225.

J. Appl. Ichthyol. 1
© 1995 Blackwell
ISSN 0175-8659

The applic

By C. (

Department of
'Department of

The measurement
enabled the develo
of individually nun
to standard nutritio
experiment. Analy:
consumption-grow
differences in appe
establish consump
consumption rates
factors, such as abs
and protein turnov
between fish in the
compare the growt

The measuremen
graphic method
several aspects o
and JOBLING 199
(e.g. CARTER et
used to measure
growth performa
odology, accura
(MCCARTHY et a
individual consu
compare the gr
characteristics. A
nutrition trials w
laboratory.

In nutritional st
importance that
calculate consum
diets. For examp
may result in vari